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Rupturing of Biological Spores As a Source of Secondary Particles in Amazonia

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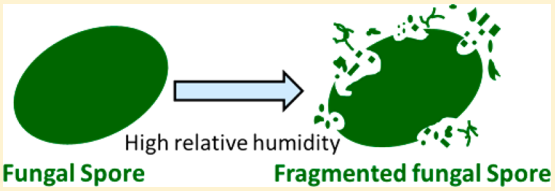
Supporting Information

ABSTRACT: Airborne biological particles, such as fungal spores and pollen, are ubiquitous in the Earth's atmosphere and may influence the atmospheric environment and climate, impacting air quality, cloud formation, and the Earth's radiation budget. The atmospheric transformations of airborne biological spores at elevated relative humidity remain poorly understood and their climatic role is uncertain. Using an environmental scanning electron microscope (ESEM), we observed rupturing of Amazonian fungal spores and subsequent release of submicrometer size fragments after exposure to high humidity. We find that fungal fragments contain elements of inorganic salts (e.g., Na and Cl). They are hygroscopic in nature with a growth factor up to 2.3 at 96% relative humidity, thus they may potentially influence cloud formation. Due to their hygroscopic growth, light scattering cross sections of the fragments are enhanced by up to a factor of 10. Furthermore, rupturing of fungal spores at high humidity may explain the bursting events of new particle formation in Amazonia.



INTRODUCTION

Aerosolized biological particles significantly influence the biosphere, atmosphere, and public health.[1−4] Biological particles impact cloud dynamics and hydrological cycles by forming clouds and ice crystals.[1,5−7] They influence the Earth's energy budget by scattering and absorbing solar radiation.[1,8] Primary biological particles, emitted directly from the biosphere, are pollen, fungal spores, bacteria, and fragments of plants and living organisms. High discrepancies exist in estimation of primary biological particle emissions,[1,9,10] ranging between 56 and 1000 Tg yr^{-1}. Aircraft and balloon measurements suggest that they can be transported to high altitudes and over long distances.[2,11−13] The physical dimensions of atmospheric biological particles span several orders of magnitude with diameters ranging from nanometers (e.g., viruses) to hundreds of micrometers (e.g., pollen grains, plant debris). Fungal spores and their fragments are one of the most abundant classes of biological particles in various environments.[2,14−16] In tropical areas, such as the Amazon basin, primary biological particles contribute up to 80% of coarse mode (2−3 μm) particle mass concentration.[2,6,17] Even at a high altitude site in North America biological particles contribute an average of 40% of the particulate organic carbon mass.[18] The global average loading and emission rates of fungal spores are ∼1 $\mu g\ m^{-3}$ and ∼50 Tg yr^{-1}, respectively.[2] Fungi can actively discharge their spores via liquid jets into the air, known as actively wet discharged spores (e.g., Ascomycota and Basidiomycota).[2,19] Concentrations of these wet discharged spores tend to increase during humid conditions such as during the wet season in the Amazon basin.[2]

Chemical compositions of primary biological particles are highly variable and remain insufficiently characterized even at a phenomenological level due to difficulties distinguishing between biological and other carbonaceous particles.[20−22] Therefore, it is currently assumed that actual contributions of primary biological particles to the total atmospheric aerosol are underestimated.[23,24] Biological materials are primarily carbonaceous and produced from metabolic activity of fungi and bacteria.[25,26] Sugar alcohols such as mannitol, arabitol, and ergosterol are commonly used as tracers for source apportionment measurements of primary biological particles, such as fungal spores.[25,26] For example, the average concentrations of mannitol were almost 2−3 times higher in the Amazon basin compared to extratropical locations.[2]

Previous studies reported that coarse pollen grains (5−150 μm) rupture under high humidity and release cytoplasmic material ranging in size from several nanometers to several micrometers.[3,4,7,27] However, these submicrometer or nanoparticles are difficult to detect by traditional bioaerosol

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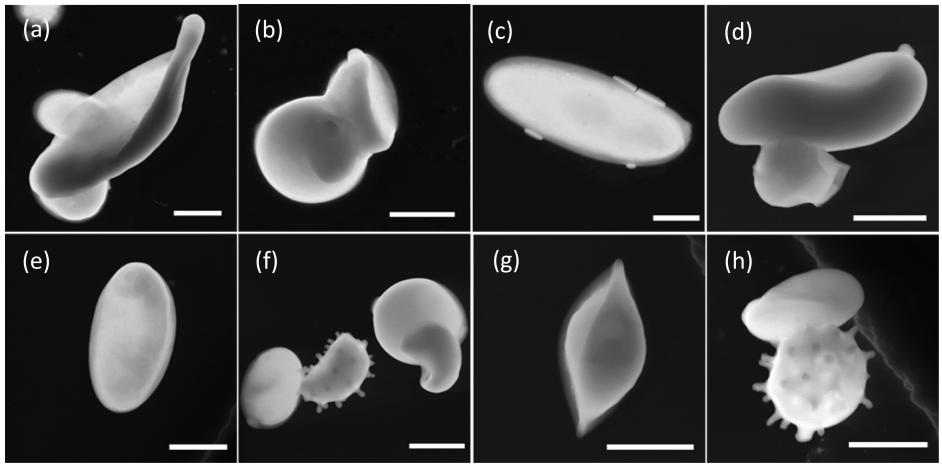

Figure 1. Representative SEM images of fungal spores collected in the Amazon basin. Scale bar is 2 μm.

sampling and analytical techniques due to their small size.[28] Pollen grains are often covered with amorphous layers and Raman spectroscopy measurements show that the chemical composition of these layers significantly differs between species.[29] Many of the fragmented submicrometer particles (subpollen) are starch granules that contribute to atmospheric organic carbon.[7,29−31] These subpollen particles can act as cloud condensation nuclei[7] and ice nuclei.[32,33] In contrast, the environmental impact and cloud formation potential of expelled particles from relatively smaller sizes (1−6 μm) fungal spores has not been recognized.

Because of the smaller size of fungal spores compared to pollens, they have longer atmospheric lifetimes and can be lofted to the mid and upper troposphere. Their abundance depends on environmental factors such as annual season, rain, thunderstorms, wind and temperature.[1,2,34,35] For example, high ambient concentrations of biological particles are associated with rainfall events.[36] Aircraft measurements over central China showed higher concentrations of fungal spore tracers in spring than in summer.[37] They have relatively high number concentrations ($\sim 10^4$ m^{-3}) in the continental boundary layer.[20] Fungal spores and other primary biological particles contribute up to 80% of coarse mode particles in the Amazon basin and significantly impact hydrological cycle.[2,17]

Here, we show evidence of rupturing of fungal spores collected in the Amazon. The rupturing and release of submicrometer subfungal fragments are observed after exposure to water vapor and subsequent drying. We discuss the previously unexplored climatic implications of these submicrometer fragments and links to new particle burst observations in central Amazonia.

EXPERIMENTAL SECTION

Samples of atmospheric particles were collected during the wet season (January and February, 2015) at the ZF2 Tower (02°35.3517′ S, 60 06.8333′ W), a pristine rainforest site in Central Amazonia located 40 km North of Manaus margins. Samples were collected from both below (~2 m above ground) and above the canopy (39 m above ground). The residence time of air parcel within the forest canopy is on the order of minutes but it can vary depending on the turbulence level. During nighttime turbulence level is low and that limit the transport of air parcel from above to within the canopy. However, during transition of nighttime to daytime, turbulence progressively increased and reaches the lower half of the canopy ~1.5 h after sunrise.[38] Particles were collected onto 400 mesh transmission electron microscopy (TEM) grids coated with Carbon Type-B films (Ted Pella, Inc.) using 10-stage Micro-Orifice Uniform Deposition Impactors (MOUDI; model 110-R, MSP, Inc.). This study focuses on the samples from stage 4 (size range: 3.2−5.6 μm) where the relative abundance of biological particles is higher than on the other stages. An environmental scanning electron microscope (ESEM, Quanta 3D model, FEI, Inc.) with a Peltier cooling stage was used for water vapor exposure experiments. Fungal spores on the substrate were investigated before water vapor exposure experiments and only intact particles were monitored during experiments. We note that potential caveats of substrate based approach cannot be avoided such as possible damage and morphology modifications of particles upon impaction. A scanning transmission electron detector was used for the ESEM imaging.[39] ESEM experiments were conducted at 278−280 K and 0.08−6.50 Torr of water vapor, corresponding to sample exposures of 1−99% relative humidity (RH). Samples were systematically exposed to high RH (~98%) for time periods of 0.5−10 h. Between exposures, samples were dried (to RH ~ 1%) by decreasing the water vapor pressure (to 0.1 Torr) of the ESEM chamber for an interval of 1−2 h. In this study a total of eight samples were investigated. Samples from above and below canopy were collected simultaneously for same duration at the same location. Table S1 (Supporting Information (SI)) shows the sampling time, duration and duty cycle. After prolonged exposure to water vapor, fungal spores ruptured and released submicrometer fragments. We dried the sample inside the ESEM chamber after rupturing of fungal spores. Later we characterized the fragments in dry condition to determine their elemental compositions and morphologies. Furthermore, we conducted dynamic hydration experiments using ESEM to study the hygroscopic behavior and growth of fungal fragment particles. Hydration experiments were conducted up to 96% RH (6.32 Torr of water vapor). Computer-controlled scanning electron microscopy with energy-dispersive X-ray (CCSEM/EDX)[40] was used to investigate the morphology and elemental composition of biological particles. TEM imaging and electron energy loss spectroscopy (EELS) measurements were performed in scanning mode using an aberration-corrected transmission electron microscope (FEI, Inc. model Titan 80−300) operated at 300 kV. Scanning transmission X-ray

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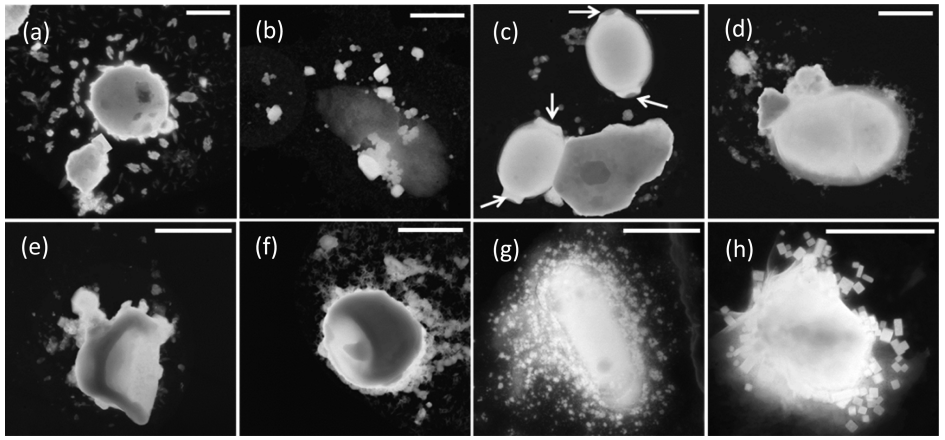

Figure 2. Representative SEM images of ruptured fungal spores and expulsion of submicrometer fragments. Panel (c) shows an example of germination pores of fungal spores as indicated by arrows. Scale bar is 2 μm.

microscopy (STXM)[41] was used to map specific elements (e.g., carbon, sodium) within individual biological particles prior to and post exposure to high relative humidity and subsequent drying.

Furthermore, we used size distribution data from a scanning mobility particle sizer (custom-built SMPS system, Lund University) collected at the ZF2 site during the wet season (Jan-Jun) in the years of 2008−2009, comprising measurements from 133 days[42] to investigate observed bursting events of nanoparticles in the Amazon basin. Particle size distribution measurements were performed 10 m above the canopy top. All measurements were performed under dry conditions (RH 30−40%), assured by an automatic diffusion dryer.[43]

RESULTS AND DISCUSSION

Size Distribution and Chemical Composition of Fungal Spores. Typically, fungal spores are a few micrometers in size, often spherical, rod-like or spheroidal (prolate) in shape.[17,44,45] Figure 1 shows representative morphologies of fungal spores found in the Amazonian samples. Spheroidal fungal spores are 3−7 μm long and 2−4 μm wide. Sizes (area equivalent diameters) of the fungal spore particles range from 1.1 to 5.9 μm with an average diameter of 2.8 μm. Previous studies found presence of several species of fungal spore in the Amazon rainforest canopy with different sizes.[46,47] These studies applied culture-independent approaches to measure the composition of total and active atmospheric fungal spores over the Amazon forest canopy. An RNA-based approach was also applied to measure metabolically active microbial communities in the atmosphere. Phyla Ascomycota and Basidiomycota were most abundant in total airborne fungal communities with relative abundance of Basidiomycota over 90%. Within the Basidiomycota, Agaricomycetes were most abundant class. However, Ascomycota found to be major fraction (mean relative abundance∼ 80%) within the active community in the atmosphere over the Amazon forest canopy. Sordariomycetes (∼27%) and Lecanoromycetes (∼18%) were most abundant classes within the Ascomycota community. Overall, these studies found presence of potentially active fungi in the atmosphere, including lichen fungi (class Lecanonomycetes) and the following genera: Agaricus; Amanita; Aspergillus; Boletus; Cladonia; Lepsita; Mortierella; Puccinia; and Rhizopus.[46,47]

Elemental composition analysis by CCSEM/EDX shows that fungal spores are primarily composed of C and O. Other frequent elements included N, Na, P, K, S; some fungal spores also contain Cl and Mg. Similar elemental compositions of biological particles have been reported previously.[48] SI Figure S1 shows an example of EDX elemental maps of a typical fungal spore particle along with its corresponding EDX spectrum. The C map indicates the fungal spore contains significant carbonaceous content. Within this primary unprocessed particle, the elements are relatively homogeneously distributed, consistent with measurements of other fungal spores found in the samples. However, the fractions of different elements vary considerably among fungal spore particles (SI Figure S2).

In this study, fungal spores were identified based on their characteristic shapes and chemical composition, which includes a high carbonaceous component and the presence of other elements such as phosphorus.[17,42,49] Fungal spore particles contributed up to 56% and 17% of the total number of particles in the size range of 3.2−5.6 μm (aerodynamic diameter) below and above the canopy, respectively.

Rupture of Fungal Spores. Rupturing of fungal spores and subsequent expulsion of fungal fragments was observed after exposure to high RH conditions (∼98%) for ∼10 h and subsequent drying. Representative examples of fragmented and expelled fungal spores after wet and drying cycles are shown in Figure 2. Substantial rupturing was not observed in the samples exposed to high RH for shorter time periods that were subsequently dried. These results suggest that rupture occurred during the prolonged wet environment, likely repeated several times, and not due to surface tension forces during a single hydration/dehydration cycle.[7] Rupturing of fungal spores at high humidity may impact the culturability of the fungal spores. Hydrated fungal spores may contain significant amount of osmotically active solutions. At high humidity osmotic pressure can be developed across fungal walls and once it is high enough then spores can rupture.[50] Figure 3 shows the size (area equivalent diameter) distributions of original fungal spores and expelled fungal fragments after rupture. Here, only individual particles that did not agglomerate during the drying process were used for the analysis. Fungal spores release fungal fragments in a broad range of sizes, from 10s of nanometers up to a micrometer. Fragmented particles smaller than 10 nm may also be present, but they cannot be unambiguously detected due to the imaging limits of ESEM. This study motivates the

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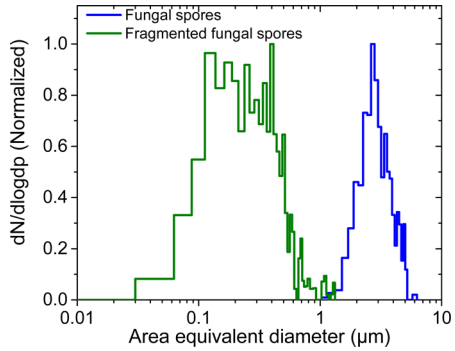

Figure 3. Size distribution of fungal spore particles (blue line) and fragmented subfungal spore particles (green line). A total of 711 individual fungal spores and 2041 fragment particles were identified by microscopy and used for the analysis.

need for further investigation of rupturing of airborne biological spores that can provide quantitative information about size and number concentration of fragments, as well as more accurate information about the environmental conditions for the rupturing process. Previous studies found that cytoplasmic material from ruptured pollen grains can be in the range of 0.05−2 μm in size.[27,34]

The size and chemical composition of fragmented particles significantly influences their cloud activation potential. ESEM images reveal that during rupture the number of particles released ranges from 10 to ~10^3 individual fragmented particles per fungal spore. Fragmented particles show distinguishable variations in their compositions and morphologies (Figure 2). In the Amazon, the presence of a variety of fungal spores with different compositions and chemical aging results in a wide range of fragmented particles. Figure 4a-c shows representative STXM images at the Na pre edge (1070 eV), peak (1078 eV) and optical density map of biological particle after exposure to high RH. Na pre edge image (Figure 4a) represents the optical density for non-Na elements over the investigated sample area and Na peak represents the maximum Na absorption. The optical density ($-\ln(I_d)/I_0$) is calculated based on the measured intensity (I_d) using Beer−Lambert's law.[51] Transmission intensity through a particle free region of the substrate is used to obtain I_0. The STXM map shows the presence of Na containing fragments and inclusions of fungal spores. Furthermore, EDX spectra of fragmented particles show signatures of inorganic salts, for example, such as Na and Cl (Figure 4d,e) and relatively low C compared to primary spore. We hypothesize that inorganic salts within the primary spore will be in liquid phase at high humidity and these salts can disperse from the primary fragments. This process may result in relatively higher inorganic salt and less carbon in the fragmented spores compared to the primary spore. A previous study reported an elemental composition of C and O in submicrometer (200 nm in diameter) fragmented pollen particles and the presence of carbohydrates and proteins in organic pollen solutions.[7]

Previously, an amorphous film around fragmented pollen was reported.[29] Chemical characteristics of the fungal spore walls and their thickness may influence their rupturing process. Water vapor can diffuse and penetrate the film thickness of the wall and facilitate the expansion of the layer, resulting in an increase of the adhesion forces, thus affecting the rupturing process.[28] Hence, understanding their wetting behavior requires characterization of the fungal spore walls. For example, pollen walls (exines) are composed of several types of proteins, lipids, and sporopollenin.[30] Previous studies found glucuronic acid, uronic acid, and heteropolymers of mannose, galactose, glucose, and glucuronic acid in several kinds of fungal spore walls.[52,53] These components and their concentrations vary among fungal spore types. The measured width of the film around the spore wall from SEM images ranged from 0.03 to 0.58 μm and increased to 0.11−0.78 μm at elevated relative humidity (SI Figure S4). Saccharide compounds in the fungal spore wall, such as glucose, can lead to the growth of the film at high RH. Similar to other carbonaceous particles, fungal spores contain high carbon, thus TEM/EELS analysis can provide further information on chemical bonding. EELS analysis shows

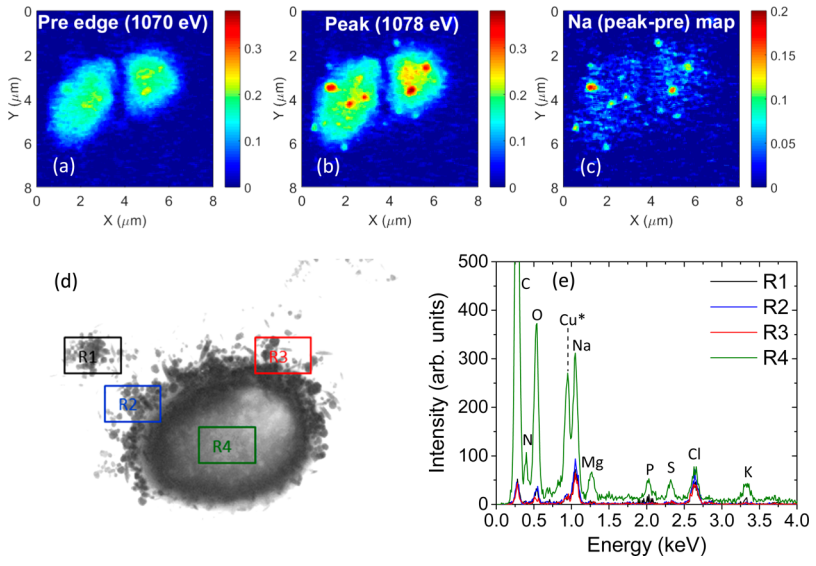

Figure 4. Representative STXM images at the (a) Na K pre-edge (1070 eV), (b) peak (1078 eV) and (c) optical density map of Na-containing biological particle (optical density difference between Na K peak and pre-edge). SEM image (d) of a ruptured fungal spore particle and its fragments after water exposure; (e) the EDX spectra acquired over different regions of the particle (marked by boxes in the SEM image) show the presence of elemental components representative of salts (i.e Na and Cl) in the fragmented particles. The Cu peak in the EDX spectrum is from the substrate.

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considerable differences in aromatic carbon (exhibited by the intensity of π^* peak) between fungal spores and carbonaceous soot particles (SI Figure S3). Fungal spores show relatively weaker π^* peak compared to soot particles. Furthermore, substantial differences in the intensity of the carbon π^* peak between various fungal spores are also noticeable. For example, our experimental observations suggest that fungal spores with weaker π^* peak (less aromatic) are more susceptible to rupture and release higher number of fragments compared to those with stronger π^* peak (SI Figure S3). Overall, TEM/EELS analysis suggests that structural variability within various fungal spores and their walls may determine the extent and specific conditions for rupturing.

Spore morphology can also influence the rupturing process and the release of materials. For example, elongated (high aspect ratio) fungal spores (e.g., Aspergillus and Penicillium) are more susceptible to stress compared to spores with less elongated structures (Cladosporium).[28] Similarly, our limited observations support the hypothesis that elongated particles are more likely to rupture than less elongated particles (Figure 2). Hence, elongated particles release a higher number of submicromter particles.

Climatic Impacts. Aircraft measurements suggest that fungal spores can be uplifted from the ground surface to the mid-troposphere[37] and potentially impact climate. In locations where the ambient relative humidity is high enough (such as the Amazon) fungal spores can rupture and release fungal fragments. Expelled submicrometer particles can potentially be transported to the free troposphere by tropical convection and further impact cloud properties. The number, mass, size, and chemical compositions of fine biological particles are insufficiently quantified and remain highly uncertain for the Amazon basin.[20−22,54] Our results suggest that expelled fungal fragments may contribute to the fine aerosol fraction. We note that temperature and ambient pollutants (e.g., O_3) during the laboratory experiments were not similar to those in the Amazon basin. Other environmental conditions (e.g., temperature, wind speed and concentrations of ambient pollutants) may also influence rupturing spores under wet conditions in the Amazon basin. However, the release of the small fungal fragments may not be easily detected by in situ techniques such as fluorescence UV-APS due to instrument detection limits.[17]

The hygroscopicity of submicrometer fungal fragments was investigated using ESEM. From ESEM images collected in the hydration experiments, area equivalent diameter growth factors ($diameter_{wet}/diameter_{dry}$) of fungal fragments were estimated in the initial (dry) size range of 150 to 600 nm (SI Figure S5). Since fungal fragments contain inorganic salt elements (e.g., Na and Cl) and fungal spore walls contain sugars, growth factors of fungal fragments are compared with those of NaCl and D-glucose. Figure 5 shows the growth factors of fungal fragments compared with hygroscopic characteristics of pure NaCl and D-glucose submicrometer particles.[55] Results suggest that the hygroscopicity of fungal fragments lies between NaCl and D-glucose particles within an RH range of 70−96%, indicating that fungal fragments are more hygroscopic than D-glucose particles. The growth factors of fungal fragments approach those of NaCl particles at high RH (~96%). The growth factors of fungal fragments are 1.18, 1.35, 1.64, and 2.31 at RH of 60, 76, 85, and 96%, respectively (Figure 5). As discussed earlier, with increasing RH, the fungal spore film wall width also increases (SI Figure S4). However, previous studies found aerodynamic diameter growth factors of 1.06−1.27 at

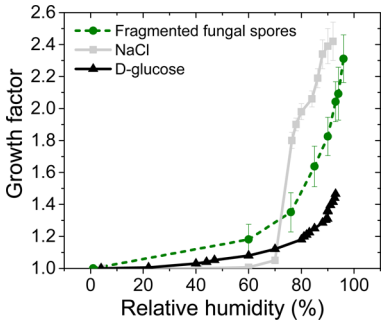

Figure 5. Area equivalent diameter growth factors ($diameter_{wet}/diameter_{dry}$) of fragmented subfungal spore particles (green line). Gray line indicates the growth factor of the laboratory generated NaCl particles. Black line indicates the growth factor of D-glucose from Mochida and Kawamura[55] using tandem differential mobility analyzer.

98% for 1.6−3.3 μm fungal spore particles.[1,56,57] Overall, our results indicate that fungal fragments are hygroscopic in nature and can participate in cloud formation. Similarly, a recent study showed that fragmented pollen grains are active as cloud condensation nuclei, requiring supersaturations of 0.81 and 0.12 for 50 and 200 nm particles, respectively.[7]

The increase in light scattering cross section due to hygroscopic growth of the fragmented particles at elevated RH can be estimated using Mie theory.[58,59] For these calculations a wavelength of 550 nm was used. The refractive indices of the hydrated particles are derived using the volume mixing rule,[60] with a real part of the refractive index of 1.40 for biological particles[48] and 1.33 for water.[61] At 60% RH, the scattering cross section of fungal fragments increased by a factor of 1.2, compared to dry conditions. The scattering cross section of fragments increases with increasing RH, meaning that a larger growth factor leads to a larger scattering cross section. The increases in scattering cross section of fungal fragments are 1.2, 1.9, 4.3, 8.6, and 10.3 at relative humidities of 60, 76, 85, and 96%, respectively (SI Figure S6). These properties could allow fragmented particles to have a direct radiative effect on climate.

Mechanisms of the new particle formation in the Amazon basin remains insufficiently understood and occurs much less frequently than reported in a boreal forest.[62] Furthermore, the Amazon basin has one of the lowest aerosol concentrations in any continental regions,[21,42] and as such the release of nano- and submicrometer particles from fungal spores can strongly influence aerosol concentration and thus its role on clouds. Nucleation events and subsequent growth of nucleated nanoparticles (3−10 nm) to larger sizes are regularly observed in other continental locations.[21,42] Bursting events of nanoparticles (SI Figure S7) in the size range of 10−50 nm occur frequently during the wet season in Amazonia.[42] However, near surface measurements revealed no significant evidence of regional scale new particle formation from gas-particle nucleation events in the Amazon basin.[21] A large amount of water vapor emitted from the forest makes the Amazon basin very humid, often >70% RH and in addition deep vertical convection is notorious in this tropical area.[42] Figure 6 shows the occurrence of bursting events in Amazon basin measured at the ZF2 site during the wet season of 2008−2009. The median diurnal variation of N_{50} concentrations (SI Figure S7 (b)) indicates that particles with diameters less than 50 nm are more frequently observed at nighttime, when ambient relative humidity reaches its highest values close to saturation (SI

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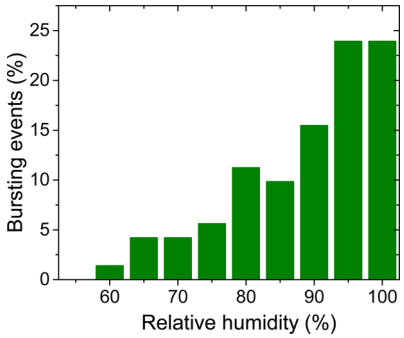

Figure 6. Bursting events in the Amazon basin at different RHs.

Figure S7 (c)) and no photochemistry occurs. Figure 6 illustrates that the occurrence of bursting events increases with increasing RH, which is in accordance with our observation of rupturing of spores at high RH. Expulsion of the fine particles from fungal spores under wet conditions in the Amazon basin, and/or outflow from deep convective clouds (in-cloud processing of fungal spores) could be common and may provide insight into new particle formation (see discussion in the SI).

The rupturing process and release of fungal fragments may impact the complex land−atmosphere exchange process (emissions and deposition). The lifetime of the fungal spore particles can increase after their rupturing and release of submicrometer fragments. This process potentially affects the deposition of biological particles. Deposition of biological particles may influence metabolic activity and trigger reproduction of biological spores. These activities can either facilitate or hinder further emission of biological particles, ultimately may affect biogeochemical cycle and related feedback loops.[63] Finally, it may alter the feedback loops of the exchange processes between the atmosphere and soil, and long-range transport of particles. Furthermore, release of compounds from the biological particles by a rupturing process is typically ignored,[2] but can substantially influence budget calculations of biological particles. Future studies should focus on (1) detailed characterization and improved understanding of the chemical composition of different types of fungal spores and the variability of their fragments; (2) systematic evaluation of specific environmental conditions promoting rupturing events (e.g., RH, temperature, atmospheric pressure, humidity cycling rates).

■ ASSOCIATED CONTENT

Supporting Information

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Additional information as noted in the text (PDF)

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Notes

The authors declare no competing financial interest.

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